

CLAIMS

- 1) Isolated single- or double-stranded DNA sequence, represented in Figure 2 (direct or complementary sequence of SEQ ID No.1) corresponding to the eryG-eryAIII region of the cluster of erythromycin biosynthesis genes.
- C* 2) ~~A DNA of claim 42 comprising~~ ~~a DNA sequence according to claim 1 comprising:~~
- the eryBII sequence corresponding to ORF7 (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046) and coding for a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
 - the eryCIII sequence corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) and coding for a desosaminyltransferase and
 - the eryCII sequence corresponding to ORF9 (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) and coding for a dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase.
- Ins C²* 3) ~~Isolated DNA sequence represented in Figure 2 chosen from~~ ~~the eryBII sequence corresponding to ORF7 (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046), the eryCIII sequence corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or the eryCII sequence corresponding to ORF9 (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) and the sequences which hybridize and/or display significant homologies with this sequence or fragments of the latter and having the same function.~~
- C* 4) ~~An isolated DNA sequence eryCII represented in Figure 2 corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308 = complementary sequence of SEQ ID No.4) and coding for a desosaminyl transferase.~~
- C* 35 5) Polypeptide coded by one of the DNA sequences according to ~~claim 42~~ ~~one of claims 1 to 4.~~
- Ins C³* 6) Polypeptide according to claim 5 corresponding to an ORF represented in Figure 2, chosen from ORF7 (having the sequence of SEQ ID No.2), ORF8 (having the sequence of SEQ ID

No.5) or ORF9 (having the sequence of SEQ ID No.3) and the analogues of this polypeptide.

7) Polypeptide according to claim 5 corresponding to ORF8 represented in Figure 2 (having the sequence of SEQ ID No.5)

5 and having a desosaminyltransferase activity, called EryCIII.

8) Isolated DNA sequence represented in Figure 3 (sequence of SEQ ID No.6) corresponding to the eryAI-eryK region of the cluster of erythromycin biosynthesis genes.

9) DNA sequence according to claim 8 comprising:

10 - the eryBIV sequence corresponding to ORF13 (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207) and coding for a dTDP-4-keto-L-6-desoxyhexose 4-reductase,

- the eryBV sequence corresponding to ORF14 (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454) and coding

15 for a mycarosyltransferase,

- the eryCVI sequence corresponding to ORF15 (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220) and coding for a dTDP-D-6-desoxyhexose 3-N-methyltransferase,

- the eryBVI sequence corresponding to ORF16 (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide 4837) and coding for a dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,

- the eryCIV sequence corresponding to ORF17 (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039) and coding for a dTDP-D-6-desoxyhexose 3,4-deshydratase,

25 - the eryCV sequence corresponding to ORF18 (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) and coding for a dTDP-D-4,6-didesoxyhexose 3,4-reductase and

- the eryBVII sequence corresponding to ORF19 (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) and

30 coding for a dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.

10) Isolated DNA sequence represented in Figure 3 chosen from the eryBIV sequence corresponding to ORF13 (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207), the eryBV sequence corresponding to ORF14 (sequence of SEQ ID No.6 from

35 nucleotide 1210 to nucleotide 2454), the eryCVI sequence corresponding to ORF15 (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), the eryBVI sequence corresponding to ORF16 (sequence of SEQ ID No.6 from

nucleotide 3308 to nucleotide 4837), the eryCIV sequence corresponding to ORF17 (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), the eryCV sequence corresponding to ORF18 (sequence of SEQ ID No.6 from 5 nucleotide 6080 to nucleotide 7546) or the eryBVII sequence corresponding to ORF19 (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) and the sequences which hybridize and/or display significant homologies with this sequence or fragments of the latter and having the same 10 function.

11) Isolated DNA sequence eryBV represented in Figure 3 corresponding to ORF14 (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454) and coding for a mycarosyltransferase.

15 12) Polypeptide coded by one of the DNA sequences according to Claim 8 to one of claims 8 to 11.

13) Polypeptide according to claim 12 corresponding to an ORF represented in Figure 3, chosen from ORF13 (having the sequence of SEQ ID No.7), ORF14 (having the sequence of SEQ 20 ID No.8), ORF15 (having the sequence of SEQ ID No.9), ORF16 (having the sequence of SEQ ID No.10), ORF17 (having the sequence of SEQ ID No.14), ORF18 (having the sequence of SEQ ID No.11) or ORF19 (having the sequence of SEQ ID No.12) and the analogues of this peptide.

25 14) Polypeptide according to claim 12 corresponding to ORF14 represented in Figure 3 (having the sequence of SEQ ID No.8) and having an mycarosyltransferase activity, called EryBV.

15) Use of at least one of the DNA sequences chosen from the sequences eryBII (complementary sequence of SEQ ID No.1 from 30 nucleotide 48 to nucleotide 1046), eryCIII (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or eryCII (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) represented in Figure 2, eryBIV (sequence of SEQ ID No.6 from nucleotide 242 to 35 nucleotide 1207), eryBV (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454), eryCVI (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), eryBVI (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide

4837), eryCIV (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), eryCV (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) or eryBVII (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156)

5 represented in Figure 3, to synthesize hybrid secondary metabolites in *Sac. erythraea*.

16) Use of at least one of the DNA sequences chosen from the sequences eryBII (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046), eryCIII (complementary

10 sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or eryCII (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) represented in Figure 2, eryBIV (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207), eryBV (sequence of SEQ ID No.6 from

15 nucleotide 1210 to nucleotide 2454), eryCVI (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), eryBVI (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide 4837), eryCIV (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), eryCV (sequence of SEQ ID No.6 from

20 nucleotide 6080 to nucleotide 7546) or eryBVII (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) represented in Figure 3 or of a fragment of this sequence, as hybridization probes.

17) Use of the eryCIII DNA sequence represented in Figure 2

25 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308 = complementary sequence of SEQ ID No.4) as a hybridization probe to isolate genes responsible for the glycosylation of the macrolactone in a macrolide-producing strain.

30 18) Use according to claim 17 in which the homologous genes are the oleandomycin biosynthesis genes in *S. antibioticus*.

19) Isolated DNA sequence represented in Figure 22 (sequence of SEQ ID No.15) corresponding to a region of the cluster of oleandomycin biosynthesis genes comprising:

35 - the sequence corresponding to ORF oleP1 from nucleotide 184 to nucleotide 1386,
- the sequence corresponding to ORF oleG1 from nucleotide 1437 to nucleotide 2714 coding for a glycosyltransferase

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activity,

- the sequence corresponding to ORF oleG2 from nucleotide 2722 to nucleotide 3999 coding for a glycosyltransferase activity,

- 5 - the sequence corresponding to ORF oleM from nucleotide 3992 to nucleotide 4720 (= sequence of SEQ ID No.20) and
- the sequence corresponding to ORF oleY from nucleotide 4810 to nucleotide 5967.

20) Isolated DNA sequence represented in Figure 22 chosen
10 from the sequence corresponding to ORF oleG1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714 coding for a glycosyltransferase activity and the sequence corresponding to ORF oleG2 (sequence of SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) coding for a glycosyltransferase
15 activity.

21) Isolated DNA sequence according to claim 20 corresponding to ORF oleG1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714) coding for a desosaminyltransferase activity.

20 22) Isolated DNA sequence according to claim 20 corresponding to ORF oleG2 (sequence of SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) coding for an oleandrosyltransferase activity.

25 23) Polypeptide coded by the DNA sequence corresponding to ORF oleG1 and having a desosaminyltransferase activity (sequence of SEQ ID No.17).

24) Polypeptide coded by the DNA sequence corresponding to ORF oleG2 and having an oleandrosyltransferase activity (sequence of SEQ ID No.18).

30 25) Process for the preparation of hybrid secondary metabolites in *Sac. erythraea* in which:

- a DNA sequence is isolated containing at least one eryB sequence or one eryC sequence of the cluster of erythromycin biosynthesis genes represented in Figure 2 (complementary sequence of SEQ ID No.1) or in Figure 3 (sequence of SEQ ID No.6),
- a modification is created in said sequence and an altered sequence is obtained,

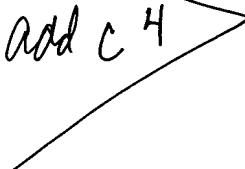
- the altered sequence is integrated into the chromosome of the host strain and a modified strain is obtained,
 - the modified strain is cultured under conditions which allow the formation of the hybrid secondary metabolite and
- 5 - the hybrid secondary metabolite is isolated.
- 26)** Process according to claim 25 in which the DNA sequence codes for one of the enzymes chosen from a
- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
 - desosaminyltransferase,
- 10 - dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
- dTDP-4-keto-L-6-desoxyhexose 4-reductase,
 - mycarosyltransferase,
- 15 - dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- dTDP-4-keto-L-6-desoxyhexose 2,3-deshydrtatase,
 - dTDP-D-6-desoxyhexose 3,4-deshydrtatase,
- 20 - dTDP-D-4,6-didesoxyhexose 3,4-reductase or
- dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.
- 27)** Process according to claim 25 in which the alteration of the sequence results in the inactivation of at least one of
- 25 the enzymes chosen from a
- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
 - desosaminyltransferase,
- 30 - dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
- dTDP-4-keto-L-6-desoxyhexose 4-reductase,
 - mycarosyltransferase,
- 35 - dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- dTDP-4-keto-L-6-desoxyhexose 2,3-deshydrtatase,
 - dTDP-D-6-desoxyhexose 3,4-deshydrtatase,
- 40 - dTDP-D-4,6-didesoxyhexose 3,4-reductase or
- dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.
- 28)** Process according to claim 27 in which the inactivated enzyme is a dTDP-4-keto-L-6-desoxyhexose 4-reductase.
- 29)** Process according to claim 27 in which the inactivated enzyme is a dTDP-D-6-desoxyhexose 3,4-deshydrtatase.
- 45 **30)** Process according to claim 27 in which the inactivated enzyme is a mycarosyltransferase.
- 31)** Process according to claim 27 in which the inactivated enzyme is a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase.

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- 32) Process according to claim 25 in which the isolated hybrid secondary metabolite is an analogue of erythromycin chosen from 4"-keto-erythromycin, 4'-hydroxy-erythromycin or 3"-C-desmethyl-2",3"-ene-erythromycin.
- 5 33) Process according to claim 25 in which the isolated hybrid secondary metabolite is desosaminyl erythronolide B.
- 34) Modified strain of *Sac. erythraea* in which at least one of the enzymes chosen from a
- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
- 10 - desosaminyltransferase,
- dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
 - dTDP-4-keto-L-6-desoxyhexose 4-reductase,
 - mycarosyltransferase,
 - dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- 15 - dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,
- dTDP-D-6-desoxyhexose 3,4-deshydratase,
 - dTDP-D-4,6-didesoxyhexose 3,4-reductase or
 - dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase
- is inactivated and producing at least one hybrid secondary
- 20 metabolite.
- 35) Modified strain of *Sac. erythraea* (BII92) in which a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase is inactivated and producing 3"-C-desmethyl-2",3"-ene-erythromycin C.
- 36) Modified strain of *Sac. erythraea* (BIV87) in which a
- 25 dTDP-4-keto-L-6-desoxyhexose 4-reductase is inactivated and producing 4"-keto-erythromycin.
- 37) Modified strain of *Sac. erythraea* (CIV89) in which a dTDP-D-6-desoxyhexose 3,4-deshydratase is inactivated and producing 4'-hydroxyerythromycin D.
- 30 38) Modified strain of *Sac. erythraea* (BV88) in which a mycarosyltransferase is inactivated and producing desoaminylerithronolide B.
- 39) Preparation process for precursors of oleandomycin in *S. antibioticus* in which
- 35 - an alteration is created in the sequence of the gene chosen from the DNA sequence corresponding to ORF *oleG1* (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714) and the DNA sequence corresponding to ORF *oleG2* (sequence of

SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) in the chromosome of a host strain and a modified strain is obtained,

- the modified strain is cultured under conditions allowing
 - 5 the accumulation of the precursors of oleandomycin and
 - these precursors are isolated.
- 40)** Process according to claim 39 in which the alteration is created in the DNA sequence corresponding to ORF *oleG1* (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 10 2714) and the result of which is at least the elimination of the desoaminyltransferase activity and the accumulation of the precursor of oleandomycin 8,8a-desoxyoleandolide.
- 41)** Thymidine 5'-(trihydrogen diphosphate), P'-[3,4,6-tridesoxy-3-(dimethylamino)-D-xylo.-hexopyranosyl] ester 15 (dTDP-D-desosamine) and the addition salts with bases.

Add C'  *Add C⁴* 

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